

# Microbial Characteristics of Vertisol Under Different Fertilization Systems

## Mikrobiološke karakteristike smonice u uslovima primene različitih sistema đubrenja

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### Abstract

The present study evaluates the effect of increasing rates of mineral nitrogen (90; 120; 150 kg×ha<sup>-1</sup>), liquid manure (80 t×ha<sup>-1</sup>) and solid manure (45 t×ha<sup>-1</sup>) on the microbial properties of vertisol (total number of microorganisms, numbers of actinomycetes and cellulolytic microorganisms) under maize.

The research results showed dependence of the number of the test groups of microorganisms on the type and rate of fertilization, as well as on the date and zone of sampling. Namely, lower nitrogen fertilization rates (90 and 120 kg×ha<sup>-1</sup>) induced a significant increase in the total number of microorganisms, whereas the high rate (150 kg×ha<sup>-1</sup>) had a depressive effect, especially in maize edaphosphere. However, the numbers of actinomycetes and cellulolytic microorganisms were not reduced under these treatments. The organic fertilizers applied had a stimulatory effect on the above soil biological parameters.

**Keywords:** microorganisms, soil, mineral fertilizers, organic fertilizers, maize

### Rezime

U radu je prikazan uticaj rastućih doza mineralnog azota (90; 120; 150 kg×ha<sup>-1</sup>), tečnog (80 t×ha<sup>-1</sup>) i čvrstog stajnjaka (45 t×ha<sup>-1</sup>) na mikrobiološke karakteristike smonice (ukupna brojnost mikroorganizama, brojnost aktinomiceta i celulozinskih mikroorganizama), pod kukuruzom.

Rezultati istraživanja pokazuju da brojnost ispitivanih grupa mikroorganizama zavisi od vrste i doze primenjenih đubriva, kao i od vremena i zone uzimanja uzoraka. Naime, primena nižih doza azotnih đubriva (90 i 120 kg×ha<sup>-1</sup>) izaziva značajno povećanje ukupne brojnosti mikroorganizama, dok je njegova visoka doza (150 kg×ha<sup>-1</sup>) delovala depresivno, posebno u edafosferi kukuruza. Za razliku od navedenog, brojnost aktinomiceta i celulozitskih mikroorganizama, nije snižena ni pri ovakvim tretmanima.

Primenjena organska đubriva, delovali su stimulatивно na pomenute biološke parametre zemljišta.

**Ključne reči:** mikroorganizmi, zemljište, mineralna đubriva, organska đubriva, kukuruz

## Detaljan rezime

U nizu agrotehničkih mera, posebna pažnja se posvećuje blagovremenoj i pravilnoj primeni različitih đubriva, kako bi njihov efekat došao do punog izražaja. Najčešća istraživanja u ovoj oblasti su usmerena u pravcu povećanja prinosa gajenih kultura, dok se baza njihovog kumulativnog dejstva (promene bioloških i hemijskih svojstava zemljišta) često zanemaruje. Zbog toga je racionalno i efikasno korišćenje mineralnih i organskih đubriva moguće samo na osnovu kompleksnog prilaza u kome važno mesto pripada mikrobiološkim istraživanjima.

Stim u vezi u ovom radu je prikazan uticaj rastućih doza mineralnog azota, tečnog i čvrstog stajnjaka na mikrobiološke karakteristike smonice (ukupna brojnost mikroorganizama, brojnost aktinomiceta i celuloliznih mikroorganizama), pod kukuruzom.

Ogled je postavljen 2001. godine, na Oglednom dobru Agronomskog fakulteta u Čačku, na zemljištu tipa smonica po sistemu slučajnog blok rasporeda u četiri ponavljanja. Kao test biljka korišćen je kukuruz NSSC-640. Ispitivane su sledeće varijante đubrenja:  $N_1PK$  ( $90:75:60 \text{ kg} \times \text{ha}^{-1}$ );  $N_2PK$  ( $120:75:60 \text{ kg} \times \text{ha}^{-1}$ );  $N_3PK$  ( $150:75:60 \text{ kg} \times \text{ha}^{-1}$ ); čvrsti stajnjak ( $45 \text{ t} \times \text{ha}^{-1}$ ) i tečni stajnjak ( $80 \text{ t} \times \text{ha}^{-1}$ ). Navedene doze azota, fosfora i kalijuma dodate su predsetveno u obliku uree, superfosfata i KCl. Organska đubriva su zaorana 10 dana pred setvu kukuruza. Mikrobiološke analize su obuhvatile određivanje ukupne brojnosti mikroorganizama, brojnosti aktinomiceta i celuloliznih mikroorganizama u edafosferi i rizosferi tokom tri vegetaciona perioda kukuruza (faza intenzivnog porasta biljke, faza mlečno voštane zrelosti i faza pune zrelosti zrna).

Rezultati istraživanja pokazuju da brojnost ispitivanih grupa mikroorganizama zavisi od vrste i doze primenjenih đubriva, kao i od vremena i zone uzimanja uzoraka. Naime, primena nižih doza azotnih đubriva ( $90$  i  $120 \text{ kg} \times \text{ha}^{-1}$ ) izaziva značajno povećanje ukupne brojnosti mikroorganizama, dok je njegova visoka doza ( $150 \text{ kg} \times \text{ha}^{-1}$ ) delovala depresivno, posebno u edafosferi kukuruza. Za razliku od navedenog, brojnost aktinomiceta i celulolitskih mikroorganizama, nije snižena ni pri ovakvim tretmanima.

Primenjena organska đubriva delovali su stimulatивно na pomenute biološke parametre zemljišta. Vegetacijski gledano, dejstvo tečnog stajnjaka slično je dejstvu visokih doza azota, odnosno, njegovo najizraženije stimulatивно dejstvo ispoljeno je krajem vegetacije, kada je biljka u najvećem procentu iskoristila lako hidrolizovani oblik azota, koji je u početku, zbog svoje visoke doze, onemogućio brzo razmnožavanje navedenih grupa mikroorganizama.

## Introduction

Of the whole range of cultural practices employed (ranging from traditional ones to the ones currently used), specific mention should be made of proper and timely fertilization, which is essential to achieving desired effects. Research trends in this field are generally focused on increasing the yield of agricultural crops, with the basis of their cumulative impact (changes in both biological and chemical properties of the soil) being often disregarded. Therefore, an efficient cost-effective use of mineral (particularly nitrogen) and organic fertilizers can be attained only if a holistic approach is adopted and if the importance of microbiological studies is highlighted.

A change in the total number of certain systematic and physiological groups of soil microorganisms can be used as a parameter in determining optimal fertilization rates to be applied in crop nutrition (Acosta and Tabatabai, 2000; Ulea, et al., 2006).

The narrowing of the C to N ratio by nitrogen fertilizer incorporation facilitates and favors mineralization processes, favorably affecting the total number and activity of soil microorganisms and therefore the content of available nitrogen compounds (Zhang, et al., 2006). The intensity of the processes is primarily dependent on the rate and type of nitrogen fertilizers. However, the N-induced increase in soil hydrolytic ability can result in degradation of soil physical and chemical properties, and hence in a multitude of serious environmental disruptions (Barabasz, et al., 2002), which suggests that this dimension of fertilizer use should be given careful attention.

The above problems can be overcome by partial substitution of these fertilizers with microbiological and organic ones. This will help improve soil physico-chemical and biological properties and facilitate the introduction of certain amounts of other nutrients, phytohormones, enzymes and some useful microorganisms (Slimek, et al., 1999). In contrast, the uncontrollable use of organic fertilizers, particularly that of liquid manure, may even induce adverse effects on the biocenosis and cultivated plants (Mandić, et al., 2004).

The objective of the research was to evaluate the effect of increasing rates of mineral nitrogen and liquid and solid farmyard manures on the microbiological properties of vertisol (total number of microorganisms, actinomycetes and cellulolytic microorganisms).

## Material and Methods

The experiment was set up at the experimental field of the Faculty of Agronomy-Cacak, during 2001, on vertisol, using a randomized block design with four replications. The test soil was characterized by acid reaction (pH 5.01), a medium supply of nitrogen and humus (0.134% and 2.68%, respectively), a good supply of readily available potassium (0.26 mg g<sup>-1</sup> soil) and a low phosphorus content (0.03 mg g<sup>-1</sup>).

The plot size was 21.25 m<sup>2</sup>, and the spacing employed - 0.5 m, i.e. 1 m between the blocks.

The trial included the following fertilization treatments (Factor A): unfertilized control; N<sub>1</sub>PK (90:75:60 kg/ha); N<sub>2</sub>PK (120:75:60 kg×ha<sup>-1</sup>); N<sub>3</sub>PK (150:75:60 kg×ha<sup>-1</sup>); solid manure (45 t×ha<sup>-1</sup>) and liquid manure (80 t×ha<sup>-1</sup>). The above nitrogen, phosphorous and potassium rates were applied prior to the incorporation of urea, superphosphate and KCl. Organic fertilizers were ploughed under 10 days before maize (NSSC-640) sowing.

Microbiological analysis involved determination of the total number of microorganisms, actinomycetes and cellulolytic microorganisms in both edaphosphere and rhizosphere (Factor B), during three different maize growing periods (I- intensive plant growth; II- milk-wax stage; III – full wax ripeness of grain). The total number of microorganisms was determined by growth on medium (Govedarica and Jarak, 1993) with 10<sup>-6</sup> of soil dilution, that of actinomycetes - by growth on Krasilnikov's synthetic agar with 10<sup>-4</sup> of soil solution, and the total number of cellulolytic microorganisms - by growth on Wacksman-Carey medium with 10<sup>-5</sup> of soil solution (Govedarica and Jarak, 1993).

The results obtained were subjected to a three-factor 6x2x3 analysis of variance (fertilizer ´ sampling zone ´ vegetation stage), and the testing of the significance of difference between individual and interactive environments was performed by t-test.

## Results and Discussion

The analysis of variance of the data obtained (Tables 1, 2 and 3) revealed that all factors studied (fertilizer type, sampling zone, maize vegetation stage) had a highly significant effect on the tested parameters of soil biological productivity. The parameters were used to assess statistical significance of their interaction.

The lower nitrogen fertilization rates (90 and 120 kg×ha<sup>-1</sup>) induced a significant increase in the total number of microorganisms, whereas the high rates (150 kg×ha<sup>-1</sup>) had a depressive effect, particularly in the maize edaphosphere (Tab. 1). The increase in the total number of microorganisms under such conditions can be associated with the intensity of mineralization processes occurring in the soil (Belinska, 1999). Organic fertilizers (both solid and liquid manures) also had a stimulatory effect on the total number of microorganisms in the soil. This was particularly true of solid manure. This finding is consistent with the results of other authors who attribute the increase in microbial numbers after organic fertilizer application to improved soil physical and chemical properties and higher levels of organic matter and beneficial microorganisms in the soil (Hoflich, et al., 2000).

Tab.1. Total number of microorganisms ( $10^{-6}g^{-1}$  of absolutely dry soil)

A		Control		N <sub>1</sub> PK		N <sub>2</sub> PK		N <sub>3</sub> PK		Solid manure		Liquid manure		$\bar{X}$	
B		Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.		
Periods (C)	I	22.0	24.3	35.0	40.3	40.0	44.3	24.3	26.3	24.6	47.3	25.0	37.0	32.53	
	II	24.0	40.0	29.3	48.0	40.3	55.0	11.3	19.3	28.3	50.3	19.3	44.3	34.12	
	III	39.3	46.3	47.3	54.3	42.3	52.3	20.0	47.3	60.6	67.0	45.3	55.6	48.13	
$\bar{X}$		32.55		42.28		45.67		24.72		46.38		38.94			
$\bar{X}$		Edaphosphere		31.47											
		Rhizosphere		42.81											
Lsd															
Lsd		A		B		C		A'B		A'C		B'C		A'B'C	
0.05		1.94		1.05		1.27		2.75		3.36		1.78		4.77	
0.01		2.56		1.39		1.68		3.64		4.45		2.36		6.31	

Ed. – Edaphosphere; Rh. – Rhizosphere

A – fertilizers applied; B – sampling zone; C – growing season

Total numbers of microorganisms increased during the maize growing period, reaching the highest value towards the end of the season, due to environmental factors (primarily temperature and humidity) affecting the abundance of microorganisms in the soil. This was experimentally confirmed by the results of Jemcev and Djukic (2000) on the effect of increasing nitrogen rates at different humidity and air temperatures on the total numbers of bacteria, fungi and actinomycetes.

The test fertilizers differed in the effects they produced during the maize growing season (Fig. 1). Namely, the most pronounced stimulatory effect was recorded with lower application rates of nitrogen fertilizers (90 and 120 kg×ha<sup>-1</sup>) at initial stages of maize development. Organic fertilizers produced this effect at the end of the growing period. The effect of liquid manure was similar to that of high nitrogen rates, with initial depression being compensated for at the end of the season, when readily hydrolyzed nitrogen was uptaken by the plant the most.

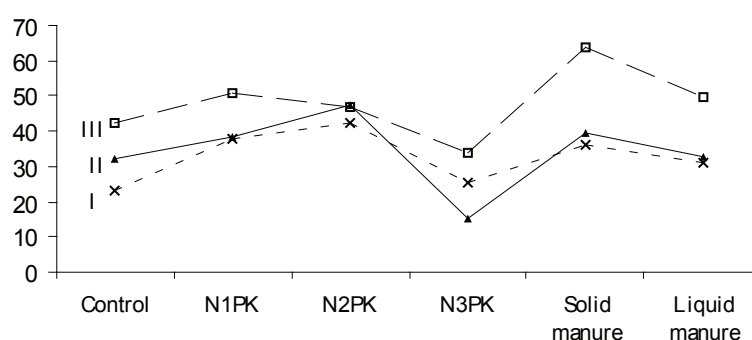


Fig.1. Interaction between the fertilizers applied (A) and the maize growing season (C) and the effect on the total number of microorganisms ( $10^6 \text{ g}^{-1}$  of absolutely dry soil), regardless of soil zone

All three nitrogen rates induced an increase in actinomycete numbers during the first period of the study. Stimulatory effects were also observed in the third period of the study in the treatment with the lowest nitrogen rate and in that with  $120 \text{ kg ha}^{-1} \text{ N}$  in the rhizosphere zone of the soil. In this soil zone, the highest nitrogen fertilizer rate was found to have the highest depressive effect towards the end of the growing season. The most pronounced stimulatory effect was exhibited by liquid and solid manures. (Tab.2).

Tab. 2. Number of actinomycetes ( $10^4 \text{ g}^{-1}$  of absolutely dry soil)

A		Control		N <sub>1</sub> PK		N <sub>2</sub> PK		N <sub>3</sub> PK		Solid manure		Liquid manure		$\bar{X}$		
B		Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.			
Periods (C)	I	14.33	19.3	13.3	25.6	20.3	27.3	18.0	26.0	29.6	40.3	27.0	25.3	23.85		
	II	10.0	16.6	10.6	9.3	7.6	9.3	8.3	7.3	15.3	15.6	18.3	19.3	12.29		
	III	30.3	41.3	43.0	55.7	31.3	50.6	33.6	30.6	75.6	70.3	79.6	75.6	51.46		
$\bar{X}$		21.89		26.33		24.33		20.33		41.03		42.56				
$\bar{X}$		Edaphosphere			25.68											
		Rhizosphere			31.05											
Lsd																
Lsd				A		B		C		A'B		A'C		B'C	A'B'C	
0.05				2.18		1.19		1.39		3.17		3.88		2.08		5.50
0.01				2.88		1.59		1.83		4.19		5.13		2.75		7.28

Ed. – Edaphosphere; Rh. – Rhizosphere

A – fertilizers applied; B – sampling zone; C – growing season

The highest numbers of actinomycetes were recorded at the end of the growing season in all treatments, and the lowest in the middle of the season (Fig. 2).

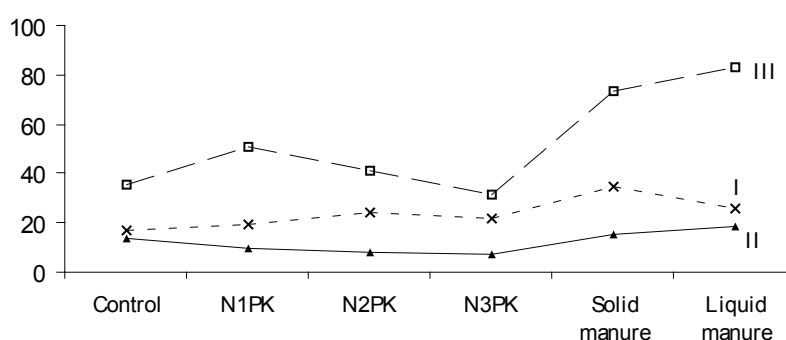


Fig.2. Interaction between the fertilizers applied (A) and the maize growing season (C) and the effect on the number of actinomycetes in the soil ( $10^4 \text{ g}^{-1}$  of absolutely dry soil), regardless of soil zone

The most pronounced effect on cellulolytic microorganisms was produced by solid manure and, proportionally to the rates applied, by mineral nitrogen (Tab.3), being in conformity with the results of other authors (Mandić, et al., 2004). The total number of these microorganisms did not decline at extreme temperatures and low soil humidity (Fig. 3), which was due to the high osmotic potential of the cells in the microorganisms analyzed (Jemcević and Djukić, 2000), as well as due to competition with other microorganisms (Franz, 1973). Furthermore, the results also suggested that low and medium nitrogen rates and solid manure rates exhibited a stimulatory effect in the first two periods, but no effect at the end of the growing season, as confirmed by the results obtained by some other authors (Koper and Piotrowska, 2003).

Tab.3. Number of cellulolytic microorganisms ( $10^5 \text{ g}^{-1}$  of absolutely dry soil)

	A	Control		N <sub>1</sub> PK		N <sub>2</sub> PK		N <sub>3</sub> PK		Solid ma- nure		Liquid manure		$\bar{X}$
	B	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	
Periods (C)	I	9.3	13.3	14.3	33.0	28.7	37.0	12.6	17.3	21.0	30.0	8.3	17.3	20.17
	II	11.0	20.0	25.3	28.3	29.0	35.3	11.6	22.6	33.6	39.7	24.6	29.3	25.85
	III	21.6	36.7	40.3	46.7	25.3	29.6	21.3	30.3	37.7	41.7	30.3	35.6	30.09
$\bar{X}$		18.66		31.33		30.83		19.33		34.05		24.16		
$\bar{X}$		Edaphosphere			21.25									
		Rizosphere			28.62									
Lsd														
Lsd		A		B		C		A'B		A'C		B'C		A'B'C
0.05		2.26		1.21		1.48		3.19		3.90		2.09		5.52
0.01		2.98		1.60		1.96		4.22		5.16		2.77		7.31

Ed. – Edaphosphere; Rh. – Rhizosphere

A – fertilizers applied; B – sampling zone; C – growing season



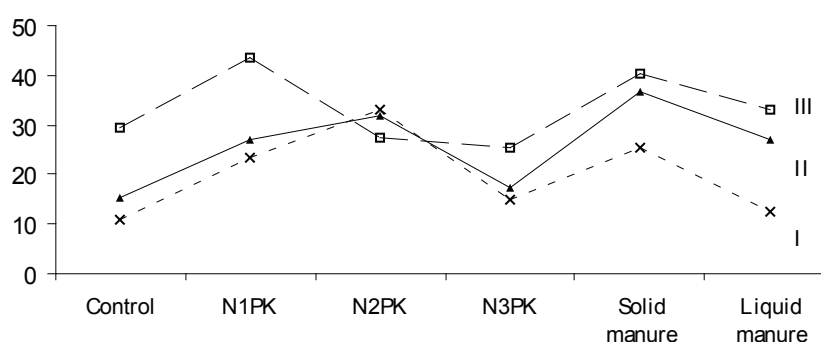


Fig. 3. Interaction between the fertilizers applied (A) and the maize growing season (C) and the effect on the number of cellulolytic microorganisms in the soil ( $10^5 \text{ g}^{-1}$  of absolutely dry soil), regardless of soil zone

## Conclusion

Total numbers of the test groups of microorganisms are dependent upon the type and rate of fertilization, as well as upon the time and zone of sampling;

Lower nitrogen fertilization rates ( $90$  and  $120 \text{ kg} \times \text{ha}^{-1}$ ) induced a significant increase in the total number of microorganisms, whereas the high rate ( $150 \text{ kg} \times \text{ha}^{-1}$ ) had a depressing effect, primarily in the maize edaphosphere. As opposed to this, the total number of actinomycetes and particularly that of cellulolytic microorganisms did not decrease with such treatments;

Overall, the organic fertilizers applied had a stimulatory effect on the above soil biological parameters, with solid manure positively affecting the total microbial number and cellulolytic microorganisms, and liquid manure stimulating soil actinomycetes.

Total numbers of microorganisms and the number of cellulolytic microorganisms increased during the growing season. The lowest number of actinomycetes was observed in the milk wax ripening stage of maize.

The number of the test microorganisms was significantly higher in the rhizosphere as compared to the edaphosphere.

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